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Effect of pregnenolone-16α-carbonitrile (PCN) on drug response in man

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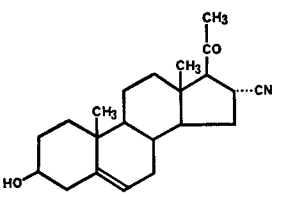
Pregnenolone-16α-carbonitrile (PCN), which alters drug responses and induces hepatic microsomal drug-metabolizing enzymes in experimental animals, is well-tolerated in man. There were no marked changes in the concentrations of blood sugar and urea; bromsulphophthalein (BSP) clearance; serum alkaline phosphatase activity; hematocrit, erythrocyte and leukocyte counts; as well as in ecg patterns. When the steroid was given by mouth for 4 days to patients treated with electroshock for psychiatric disorders, it decreased suxamethonium-induced skeletal muscle fasciculation and shortened thiopentone as well as propanidid anaesthesia. Breathing was resumed more rapidly than in control subjects. The protective effect of PCN is probably mediated through induction or activation of hepatic drug-metabolizing enzymes, or both.

Pregnenolone-16α-carbonitrile (3β-hydroxy-20-oxo-5-pregnene-16α-carbonitrile: PCN; I) was synthesized in 1958 (Romo, 1958; Ellis, Petrow & Wedlake, 1958). Its biologic activity was first reported by (Selye (1970) and Selye, Mecs & Szabo, 1970), when pretreatment of rats was found to protect them against some drugs (e.g., digitoxin, indomethacin) but not against others (e.g., sublimate). Among 500 synthetic and naturally occurring steroids of different chemical structure and pharmacologic activity, PCN was shown to be the most potent in reducing the effects of various toxic compounds (e.g., hexobarbitone, progesterone, zoxazolamine, nicotine, chlordiazepoxide, colchicine, cyclophosphamide, ethion, ethyl morphine, flufenamic acid, gluethimide, meprobamate, pancuronium, theobromine, thiopentone, tribromoethanol) (Selye, 1971). In diminishing digitoxin and indomethacin poisoning in rats, it remained the most potent among more than 800 additional steroids synthesized mainly on the basis of structure-activity correlations with PCN (Selye, Szabo & Kouronakis, unpublished observations). The esters of PCN are more active than the parent compound in some cases, probably due to their more rapid absorption.



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PCN induces hepatic microsomal (Garg, Kovacs & others, 1970; Solymoss, Werringloer & Toth, 1971; Werringloer, 1971, 1972; Kourounakis, Szabo & others, 1973; Szabo, Kourounakis & others 1974) as well extramicrosomal drug-metabolizing

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enzymes, such as, phosphoprotein phosphatase (Szabo, Selye & others, 1972). These findings have been confirmed by Somogyi, Kovacs & others (1971), Conney, Lu & others (1974), Einarsson & Gustafsson (1973), and Talcott & Stohs (1973). PCN, like phenobarbitone, increases the amount of microsomal cytochrome P-450 in the liver, but the steroid is the more potent of the two in this respect. Furthermore, in enhancing the biotransformation of drugs, PCN and phenobarbitone have different spectra of activity.

MATERIALS AND METHODS

These studies were on male and female volunteers at the Department of Psychiatry ("Dr. Gero Istvan" Medical Centre, Senta, Yugoslavia) as an adjunct to electroshock therapy during anaesthesia and skeletal muscle relaxation. No other drugs were given concurrently.

Depolarizing neuromuscular blocking agents produce transient muscular fasciculation before complete relaxation (Koelle, 1970). Hence, the following 3 parameters were measured: sleeping time after anaesthesia; resumption of breathing after electroshock; and the intensity of skeletal muscle fibrillation.

PCN was administered by mouth in capsules with lactose as a vehicle. Control patients received only lactose capsules.

In the first assay (Table 1), the compounds were administered in absolute doses. On "day 1", the patients were given 250 mg (i.v.) of thiopentone [Pentothal (Abbott Galenika, Belgrade, Yugoslavia)] with 50 mg (i.v.) of suxamethonium [Leptosuccin (Pliva, Zagreb, Yugoslavia)]. A few minutes later, when they were anaesthetized and relaxed, they were given electroshock (4 mA s^{-1}) (Elektrosok Aparat, Elektroindustrija, Nis, Yugoslavia). Resumption of breathing and awakening (ability to open eyes on command), were measured in minutes and seconds. The intensity of

Table 1. *Effect of PCN on thiopentone anaesthesia and suxamethonium muscle fasciculation in man.*

Patient	PCN*	Sleeping time		Resumption of breathing†		Muscle fasciculation (scale 0–3)	Blood Sugar (mg per 100 ml)	Blood Urea (mg per 100 ml)	BSP (% in 45 min)
		min	s	min	s				
I, f.	b	8	5	5		+++	70	24	3·2
42 y	a	4	12	3		+	70	20	6·6
60 kg									
II, f.	b	6		3		+++	94	12	12
33 y	a	3		2		+	88	12	6·5
79 kg									
III, f.	b	5	10	3	25	+++	98	20	1·1
50 y	a	4	10	1	35	+	82	12	31
51 kg									
IV, f.	b	6		4	12	+++	86	18	1·7
36 y	a	5		3	10	+	98	16	5
55 kg									
V, m.	b	6		4		+++	89	20	8·9
68 y	a	5		3		+	96	12	19·2
72 kg									

PCN, thiopentone and suxamethonium were given in absolute amounts.
* The values are expressed as: b = before and a = after PCN treatment.
† Resumption of breathing after electroshock in anaesthetized and relaxed patients.

temporary muscle fasciculation was expressed in an arbitrary scale of + to +++ where + = just detectable, ++ = moderate, and +++ = maximal fasciculation. PCN was administered as follows: 100 μg twice on the second day; 1 mg twice on the third day; 10 mg twice on the fourth day; and 10 mg three times on the fifth day. A double-blind study was performed on "day 6", after treatment as on the first day, but the measurements were taken by an independent observer. In the afternoon of the first and sixth day, venous blood was collected for measurement of glucose (*o*-toluidine, normal value 60–100 mg dl^{-1}), urea (Kowassky, normal value 20–45 mg dl^{-1}), and bromsulphophthalein (BSP, normal value 0–5% in 45 min), as well as for haematocrit, erythrocyte and leukocyte counts. Arterial blood pressure, pulse rate and ecg were also recorded.

In the *second assay* (Tables 2 and 3), PCN and the other compounds were given per kg body weight. On "day 1", the patients were administered 0·75 mg kg^{-1} of suxamethonium (i.v.) with either 6·5 mg kg^{-1} of thiopentone (i.v.) (Table 2) or 9 mg kg^{-1} of propanidid [Epontol (Bayer) (i.v.) (Table 3). They received electroshock as in the first assay. PCN was administered as follows: 50 μg kg^{-1} twice on the second day, 100 μg kg^{-1} twice on the third, fourth and fifth day. The anaesthetic agents and the muscle relaxant were given again with electroshock on the sixth day, as in the first assay. As well as the various parameters measured in the first assay, the activity of serum alkaline phosphatase (normal value 21–91 IU $litre^{-1}$ at 37° incubation) was also assessed.

During the second part of this assay, 3 patients were injected twice, at 5 day intervals, with thiopentone and suxamethonium, and 3 others with propanidid and suxamethonium at the doses in the second assay, to detect possible adaptation or habituation to these drugs.

Table 2 *Effect of PCN on thiopentone anaesthesia and suxamethonium muscle fasciculation in man.*

Patient	PCN*	Sleeping time		Resumption of breathing†		Muscle fasciculation (scale 0–3)	Blood Sugar (mg per 100 ml)	Blood Urea (mg per 100 ml)	BSP (% in 45 min)	SAP‡ (IU $litre^{-1}$)
		min	s	min	s					
VI, f.	b	10	40	8	10	+++	78	16	2·8	—
20 y	a	6	10	3	45	++	92	20	3·2	—
49 kg										
VII, f.	b	5	25	3	5	+++	78	18	3·4	22
58 y	a	6		2	50	+	81	20	2·9	24
60 kg										
VIII, m.	b	11		7	30	+++	88	10	6·1	24
22 y	a	7	45	6	5	+	84	14	2·2	20
56 kg										
IX, m.	b	7	15	4	10	+++	103	12	11·3	20
28 y	a	6	15	3	40	++	88	12	0·4	16
65 kg										
X, m.	b	10	40	6	5	+++	78	20	4·2	26
61 y	a	9	50	3	41	+	86	18	3·5	24
66 kg										

PCN, thiopentone and suxamethonium were given per kg body weight.
* The values are expressed as: b = before and a = after PCN treatment.
† Resumption of breathing after electroshock in anaesthetized and relaxed patients.
‡ Serum alkaline phosphatase.

Table 3 *Effect of PCN on propanidid anaesthesia and suxamethonium muscle fasciculation in man.*

Patients	PCN*	Sleeping time		Resumption of breathing†		Muscle fasciculation (scale 0–3)	Blood Sugar (mg per 100 ml)	Blood Urea (mg per 100 ml)	BSP (% in 45 min)	SAP‡ (IU litre^{-1})
		min	s	min	s					
XI, f.	b	6		5	50	+++	98	12	2·9	27
43 y	a	4	45	4		+	141	16	2·5	25
56 kg										
XII, f.	b	8		7		+++	98	22	0·1	27
30 y	a	6		5	40	+	71	26	3·8	20
53 kg										
XIII, f.	b	5	15	4		+++	114	14	1·7	—
27 y	a	5		3	15	++	77	32	1·8	—
56 kg										
XIV, f.	b	6	45	5	15	+++	87	20	2·2	19
46 y	a	6	45	5		+	92	18	2·6	25
51 kg										
XV, f.	b	4	50	4	10	+++	86	16	1·2	—
44 y	a	4		3	30	+	154	28	10	—
59 kg										
XVI, f.	b	5	30	5	10	+++	79	10	2·1	25
27 y	a	5	20	5		++	75	12	1·4	25
57 kg										
XVII, f.	b	5	60	4	40	+++	70	18	3·9	32
46 y	a	5		4		+	82	14	3·9	39
50 kg										
XVIII, f.	b	4	30	4		+++	81	18	2·1	32
49 y	a	5		4	45	++	78	20	0·4	39
58 kg										
XIX, f.	b	7		6	50	+++	103	16	8·7	48
51 y	a	6		6	30	+	88	12	9·6	86
70 kg										

PCN, propanidid and suxamethonium were given per kg body weight.
* The values are expressed as: b = before and a = after PCN treatment.
† Resumption of breathing after electroshock in anaesthetized and relaxed patients.
‡ Serum alkaline phosphatase.

For statistical evaluation the "Exact Probability Test" of Fisher and Yates (Finney, 1948; Siegel, 1956) was used.

RESULTS

PCN did not markedly alter the values of blood glucose, urea and BSP, nor did it significantly change the haematologic parameters, blood pressure, pulse rate and ecg.

The *first assay* (Table 1) shows that patients given absolute amounts of PCN after electroshock resumed breathing before the controls. They also slept less following thiopentone administration, and exhibited barely detectable skeletal muscle fasciculation due to suxamethonium.

The *second assay* (Table 2) provided similar results in patients given PCN per kg body weight after thiopentone, suxamethonium and electroshock. Sleeping time was not reduced in 1 subject only but, even here, skeletal muscle fasciculation was diminished and time for the resumption of breathing shortened.

The steroid decreased sleeping time in 7 out of 9 patients treated with propanidid and suxamethonium (Table 3). Breathing was resumed earlier in 8 out of 9 subjects, as compared with the controls.

The numbers of subjects in whom sleeping time was reduced and in whom resumption of breathing was hastened for the two assay groups were statistically significant:—for thiopentone and suxamethonium with and without PCN: sleeping time-control (no PCN) 0/3, PCN-treated 9/10 $P < 0{\cdot}05$; resumption of breathing-control 0/3, PCN-treated 10/10 $P < 0{\cdot}005$; for propanidid and suxamethonium with and without PCN: sleeping time-control 0/3, PCN-treated 7/9 $P < 0{\cdot}05$; resumption of breathing-control 1/3, PCN treated 8/9 $P < 0{\cdot}05$.

DISCUSSION

PCN appears to be well-tolerated in man at the doses used. This is in agreement with animal experiments where rats—given up to 100 mg of PCN twice daily by mouth for one month—were healthy and showed no signs of intoxication (Selye, 1971a; Garg, Kovacs & Tuchweber, 1972). The elevated blood glucose concentrations in 2 out of 19 patients remain unexplained but the lack of significant disturbances in haematologic and cardiac functions as well as in BSP and blood urea clearance suggests that the drug could have potential clinically.

Thiopentone anaesthesia and suxamethonium-induced muscular fasciculation were reduced when the steroid was given in absolute amounts or per kg body weight.
The effect of thiopentone was also diminished in rats by PCN pretreatment (Selye, 1971a). This barbiturate is known to undergo oxidation (replacement of S by O and partial conversion to pentobarbitone) (Williams, 1971) as well as aliphatic hydroxylation at the terminal methyl group through drug-metabolizing enzyme activity in hepatic microsomes (Daly, 1971). Since PCN, like other catatoxic steroids (e.g., spironolactone, ethyloestrenol), induces these enzymes in the rat liver and accelerates the plasma clearance of pentobarbitone through a cycloheximide or dactinomycin-sensitive process (Solymoss, Krajny & others, 1970), the reduction of thiopentone anaesthesia in man might be due to a similar action of PCN. We have no explanation for the failure of PCN to shorten thiopentone sleeping time in 1 of our 10 test subjects.

Suxamethonium is hydrolysed by plasma and liver cholinesterases. The diminished muscular fasciculation in PCN-treated patients is probably associated with enhanced suxamethonium biotransformation but other mechanisms of action cannot be excluded. PCN and some gluco- and mineralocorticoids modify the effects of various sketetal muscle relaxants and neuromuscular blocking agents, but corticoids alter drug responses through other pathways (e.g., electrolyte imbalance, drug excretion) (Szabo, Kourounakis & others 1974; Szabo, Selye & others, 1974).

In our studies, the anaesthesia produced by propanidid was also diminished by PCN treatment. Recently, propanidid was shown to be rapidly hydrolysed by serum cholinesterase and metabolized by microsomal cholinesterase (Doenicke, Kugler & others, 1973). Thus, induction of these enzyme systems by PCN may also be implicated here.

PCN increases elimination of bromosulphophthalein in rats through enhanced bile flow, glutathion conjugation and bile excretion (Zsigmond & Solymoss, 1972). We have no explanation why this effect of PCN was observed only in some of our patients.

Phenobarbitone and several other compounds (e.g., DDT, tolbutamide) exert drug metabolizing enzyme-inducing effects in man (e.g. Conney & Burns, 1972, but most inducers have undesirable side-effects (e.g., anaesthetic, toxic, anticoagulant), which interfere with their clinical use. Since PCN has no known pharmacologic actions other than enzyme induction it should be suitable in acute and chronic drug overdosage (e.g. with digitoxin, indomethacin), nonhaemolytic, non-obstructive jaundice, kernicterus and long term exposure to pesticides. In support of this is the evidence that PCN abolished signs of digitoxin and indomethacin poisoning in rats even when it was administered after drug intoxication was apparent (Selye, 1971b).

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